PRACTICAL APPLICATION OF ETHANEDINITRILE TO CONTROL INFESTED PINE WILT DISEASE AND ITS VECTOR

B.J. CHUNG[1], B.H. LEE[1,*], H. WAN [1], C.H. CHO [1,2] , J.K. SON[2] , J.K. PARK[2] ,
C.T. CHOI[3], R. Ryan[4], Y.L. REN[5]

[1] AgroLife Research Institute, Dongbu Hitec Co. Ltd, Korea
[2] IALS, Gyeongsang National University, Jin-ju 660-701, Gyeongnam, Korea
[3] NPQS, Ministry of Agriculture & Forestry, Anyang, Gyeonggi, 430-016, Korea
[4] BOC Limited [5] CSIRO, Entomology GPO Box 1700, ACT 2601, Canberra, Australia

In Korea, pine wilt disease (PWD) causes serious damages in pine forest system (Kwon et al. 2005). Currently fumigation is one of the registered practical methods to prevent spreading of the disease in Korea. Fumigation with metam sodium (MS), a methyl bromide (MeBr) alternative, controls the internal stages of vector, pine sawyer,, *Monochamus alternatus* and the pine wilt nematode, *Bursapelenchus xylophilus*. However, MS fumigation process has occupational health and safety issues with fumigation tent which is covered after spraying of liquid fumigant to felled trees. Another issue is the high label dose of MS (1L/m3) may damage soil and water environment. The new alternatives chemical, ethanedinitrile, is a timber fumigant patented by CSIRO in 1995 and is an efficacious chemical with minimal environmental problems. This field study showed the potential of ethanedinitrile to control pine sawyer and pine nematode in infested PWD pine trees in practical condition in Korea.

Materials and Methods

Wood Preparation. Pine wood was sawn from Korean red pine (*Pinus koraiensis*) that had been attacked by *Monochamus alternatus* and showed pine wilt disease (PWD). The Korean Pine tree was cut into approximately 80-90 cm diameter samples. Moisture content random selected 10 samples was measured by oven drying method (American Standard Test Methods, 1983).

Fumigants. Ethanedinitrile (purity=97%) was supplied from BOC Australia. Metam sodium (Kilper®, 25% SL) was purchased from Bayer Crop Science in Korea

Fumigation Trial of metal frame covered with polyethylene. Two field fumigation trials were conducted at the Geomjeongri Forest, Sacheon, Kyungnam Province, Korea. Fumigation chambers (1m x 1m x 1m) were constructed using metal frames covered with polyethylene. Each chamber was loaded with prepared wood samples. The calculated ethanedinitrile weight (g/m3) was injected into the chambers. During the dosing of the ethanedinitrile, the air temperature in each chamber was recorded using Thermo Recorder (TR-71U). After fumigation for 14 days, the chambers were aerated for 2 day.

Measurement of ethanedinitrile concentration. The concentration of ethanedinitrile in fumigated chamber was sampled using Tedlar bags and measured by gas chromatography [GC]. GC Analyses of ethanedinitrile was performed using a FID detector and a DB-WAX Column. The GC oven temperature was 150℃ and injector and detector temperature was 200℃. The concentration of ethanedinitrile at ambient temperature and pressure was achieved using method described by Ren et al. 2006.

\Bioassays of pine wilt nematode and pine sawyer larvae. PWN mortality was calculated post-fumigation. Random selected 10 samples of fumigated wood from each chamber was sawn in approximately 2cm thickness from the 30cm bottom of logs (diameter size over 13cm) and nematodes extracted using the modified Baermann funnel procedure (Southey, 1985). In pine sawyer larvae assay, each fumigated wood was carefully split open with hand hatchet, chisel and saw. All larvae were counted and evaluated in incubator for 72hr at 25℃±2℃. Same method was used for control mortality.

Results

Concentrations of ethanedinitrile and other factors during the fumigations

The various applied concentrations (48, 69, 97, 158g/m3) of Ethanedinitrile in the metal frame covered polyethylene for 3 days fumigation is showed in Fig.1. The concentration of ethanedinitrile was steeply reduced to one third at 6hr exposure and one tenth at the 24hr compared to initial applied dose. In this field studies, water content of fumigated pine trees averaged 55.5% for 1st trial and 68.2 % the 2nd trial... During the two fumigation, air temperature averaged 4.4℃(Max, 25.7, Min -7.0) for 1st trial and 6.1℃ (Max 23.1, Min -3.7) for 2nd trial.

Toxicity of Ethanedinitrile to pine wilt nematode and Japanese pine sawyer larvae

Ethanedinitrile was found to be effective against pine sawyer larvae when applied at 97g/m3 at very low temperature (Average 4.4 and 6.1 ℃) conditions (Table1). There is no exactly comparison. As with conduction of previous laboratory trials, increasing temperature decreased the Ct dosage of ethanedinitrile required (Ren et al. 2006) and MeBr required (Barak et al. 2005). The Ct products of ethanedinitrile treatments over a range of temperatures (4.4 to 21.1℃), 6hr exposure on *Anoplophora glabripennis* larvae had been known.

The Ct product of $L(Ct)_{99.5}$ values increased 3-6 times when the temperature increased from 4.4 to 21.1℃. This field study at relatively high temperature (not considering sorption / desorption and different water content of target pine wood), showed effectiveness of ethanedinitrile is relatively much higher. Although didn't show the Probit analysis estimated, the $L(Ct)_{99.0}$ values is estimated between 657 and 1074gh/m^3 at 5.3℃ (average temperature for two trials).

Toxicity of ethanedinitrile was not dependent on the diameters of treated logs in this experiments (P>0.05). Ethanedinitrile was highly effective (more than 95%) to PWN when applied at 97g/m^3 (Table 2). According to 2 samples test between applied dose of 48 and 69 mg/m3 test, there are no statically differences of mortality (P >0.05). The highest dose of ethanedinitrile (148g/m^3) in the trials showed highest nematocidal activity but didn't achieve 100% mortality. In field chamber fumigation of sulfuryl fluoride, Dwinell et al (2005) reported the difficulty of SF to completely control PWN even at High $L(Ct)_{100}$ values of more than 1533 gh/m^3 at high temperature (24℃). Barak et al (2005) evaluated systematic MeBr fumigation with different temperatures and doses against *A. glabripennis* larvae. At application rate of 80g/m^3 of MeBr for 24hr at 4.4℃, *insect* larvae showed 100% mortality. Comparative toxicity between MeBr and Ethanedinitrile against termite, *Reticulitermes speratus* in open and wood enclosed assay for 6hr at 21±2℃, ethanedinitrile showed several times higher efficacy than MeBr in terms of LD_{99} value (Unpublished data). However, the simple comparison between MeBr treatment (Barak et al. 2005) and ethanedinitrile in this test at 4.4℃ is too complicated to analyze because of difference of sorption factor related to wood moisture content, wood species, as well target species.

This field trial didn't measure optimal fumigation conditions in terms of low temperature. Additional fumigation trials to control PWN and its vector, JPS in Korea should schedule in low temperature [winter time / late spring] to capture the emerging adult from internal stage of pine sawyer. Based on this first field application of ethanedinitrile, there is a need for different exposure times and schedule dose with real practical temperature as MeBr alternatives.

Acknowledgements

We thank to Korea Forest Service to support this National Project of Controlling Pine Wilt Disease.

Table 1. Ethanedinitrile efficacy to Japanese Pine Sawyer larvae, *Monochamus alternatus* Larvae

Fumigant	**Dose (g/m3)**	**Range of Dia. of logs**	**No. of Treated logs**	**Mean Insect No. VS Diameter of treated logs**			**Mortality (SD)**
				Total PSL	**Alive PSL**	**Dead PSL**	
EDN	48	1	16	1.88(±0.91)	0.81(±0.83)	0.38(±0.50)	31.6
		2	16	1.88(±1.22)	0.38(±0.50)	0.81(±1.10)	68.4
		3	4	1.00(±1.41)	0.25(±0.50)	0.75(±0.95)	75.0
			N=36				58.3(±23.4)
EDN	48	1	17	1.77(±1.07)	0.65(±0.70)	0.51(±0.80)	45.0
		2	13	2.00(±1.00)	1,15(±0.80)	0.84(±1.14)	42.3
		3	7	0.86(±0.69)	0.71(±0.76)	0.14(±0.38)	16.7
			N=37				34.7(±15.6)
EDN	69	1	9	1.78(±2.39)	0.11(±0.33)	1.67(±2.12)	93.8
		2	20	1.60(±1.76)	0.20(±0.52)	1.40(±1.47)	87.5
		3	6	2.50(±2.81)	0.17(±0.41)	2.33(±2.50)	93.3
			N=35				91.5(±3.50)
EDN	69	1	10	1.80(±1.87)	0.00(±0.00)	1.80(±1.87)	100
		2	24	2.25(±2.25)	0.17(±0.48)	2.08(±1.91)	92.6
		3	3	1.00(±0.00)	0.00(±0.00)	1.00(±0.00)	100
			N=37				97.5(±4.27)
EDN	97	1	4	1.00(±1.41)	-	1.00(±1.41)	100.0
		2	24	2.00(±1.61)	-	2.00(±1.61)	100.0
		3	3	1.67(±1.15)	-	1.67(±1.15)	100.0
			N= 31				100.0(±0.0)
EDN	148	1	11	1.46(±0.82)	-	1.46(±0.82)	100.0
		2	18	1.72(±1.74)	-	1.72(±1.74)	100.0
		3	4	0.75(±1.50)	-	0.75(±1.50)	100.0
			N=33				100.0(±0.0)
MS	1000ml	1	20	1.50(±1.23)	-	1.50(±1.23)	100.0
		2	16	1.44(±1.46)	-	1.44(±1.46)	100.0
		3	4	0.75(±0.96)	-	0.75(±0.96)	100.0
			N=40				100.0(±0.0)
Untreated	-	1	10	1.00(±0.94)	1.00(±0.94)	-	0
		2	15	2.13(±1.77)	2.13(±1.77)	-	0
		3	4	1.25(±0.96)	1.25(±0.96)	-	0
			N=29				0(±0.0)

Untreated	-	1	22	0.77(±0.87)	0.77(±0.87)	-	0
		2	18	1.67(±1.34)	1.67(±1.34)	-	0
		3	4	0.25(±0.50)	0.25(±0.50)	-	0
			N=44				0(±0.0)

Range of diameter (cm) of logs: 1 (3- 6.5cm), 2 (6.6-13cm) 3 (over 13cm) PSL: pine sawyer larvae

Means of insect number (total, alive and dead insects) versus Diameter of treated logs do not differ significantly (P>0.05) in ANOVA test.

Table 2. Ethanedinitrile efficacy to PWN, *Bursapelenchus xylophilus*

Fumigant	**Dose (g/m3)**	**No. of Sample tested**	**Mean No. of Nematode per 100g of wood chips(SD)**	**95.0% CI**	**Mean Mortality (%) (SD)**
EDN	48	10	190.5(±81.9)	(131.9, 249.1)	88.43(±4.96)
	69	10	363.2((±265.6)	(173.2, 553.2)	77.94((±16.14)
	97	25	59.9((±45.5)	(41.9, 78.7)	96.36((±2.77)
	148	25	32.6((±23.5)	(22.9, 42.3)	98.02((±1.43)
MS	1000ml	25	38.8((±44.2)	(20.6, 57.1)	97.43((±2.68)
Untreated	-	60	1646((±1984)	(1133, 2159)	-

Mean Mortality: based on untreated sample

SD (Standard Deviation).

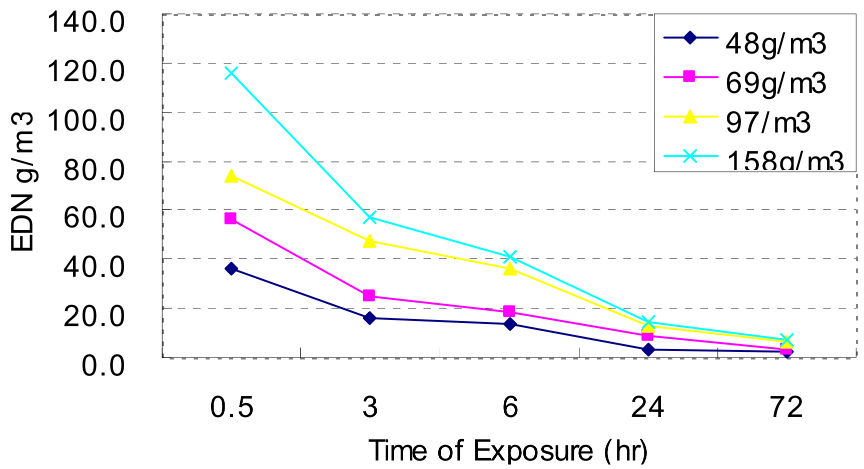

Fig. 1. The concentrations of ethanedinitrile in the metal frame covered polyethylene during the 3 days fumigation. (—◊— 48g/m3, —□— 69g/m3, —Δ— 97g/m3 and—X— 158g/m3)